# Temperature control of burying and feeding activity of *Holothuria scabra* (Echinodermata: Holothuroidea)

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## ABSTRACT

The relationships between temperature and burying and feeding behaviour of adult *Holothuria scabra* (sandfish) within a diel cycle was investigated. Animals were kept in aquaria in a constant light regime (14 h/10 h) and temperature was reduced 1°C each day from 24°C to 17°C. Burial state and behaviour (e.g. burying, feeding, resting) was scored at two-hourly intervals. Faeces were collected to investigate the relationship between sediment ingestion and temperature. A distinct diel burying and feeding cycle was exhibited, with most animals exposed and feeding between 13:00 and 20:00, and most buried and inactive between 01:00 and 09:00. Buried periods increased with decreasing temperature from 6.7 h per day at 24°C to 14.5 h per day at 17°C. Feeding activity decreased from 9.8 h a day at 24°C to 0.8 h per day at 17°C. A Generalized Linear Model showed temperature had a significant correlation with both feeding (p <0.001) and burying behaviour (p = 0.002). Faeces production also showed a statistically significant (p <0.001) relationship with temperature, and decreased from a daily production of about 40 g per day at 24°C to only 17 g per day at 17°C. Knowing when *H. scabra* will be buried is thus crucial for conducting visual population surveys for conservation and fishery research. Such surveys must have consistent diel and seasonal timing if results are to be meaningfully compared. In the southern hemisphere this would be during summer (December to February) from midday to late afternoon. ❑ *Holothuria scabra; burying; diel cycle; excretion rates; feeding; temperature; sea cucumber; sandfish*

*Holothuria scabra* Jaeger, 1833, commonly called sandfish, is one of the group of bottom dwelling holothurians. These species feed predominantly on bacteria and detritus by means of ingesting sediment and extracting organic material from it (Yingst 1976; Moriarty 1982; Baskar 1994; Conand 1999). *H. scabra* predominantly forages in the vicinity of seagrass beds, with shallow inshore waters being the preferred habitat and nursery area (Mercier *et al.* 2000a, b).

*Holothuria scabra* shows various cyclical patterns of burying depending on age (Yamanouchi 1939, 1956; Battaglene 1999; Mercier *et al.* 1999, 2000a; Uthicke 2001). Juveniles, probably due to their higher risk of predation, are synchronised by day/night regimes, burying at sunrise and re-emerging at sunset. When the juveniles reach about 40mm in length, they respond more to diel changes in temperature, by burying earlier at night (3:30) and emerging sooner during the day (12:00) (Mercier *et al.* 1999).

Studies on the burying activity of adult sandfish are scarce and fragmentary (Yamanouchi 1939, 1956; Skewes *et al.* 2000; Purcell & Kirby

2005). Yamanouchi (1939, 1956) studied *H. scabra* along with several other deposit-feeding species. However, his most detailed results about burying and feeding activity are in relation to `brown sandfish' (*Bohadschia marmorata*, then named *Holothuria vitiensis*) and he did not mention the effect of temperature on the animals. Skewes *et al.* (2000) studied *H. scabra in situ* on Warrior Reef, Torres Strait, during daylight hours and found that the animal's burying was cyclical and related to tides. However, sampling was done on only five occasions and results were highly variable depending on location and seagrass cover, and water temperature was not investigated. Purcell *et al.* (2005) were focused more on *in situ* locomotion than burying of *H. scabra*, and mentioned that adults bury during daylight only during the coldest period of the year. However, they did not specify at what temperature changes occurred.

Table 1. Glossary of terms used in this paper.

| Term | Definition |
|------|-----------|
| activity | combination of burial state and behaviour of animal |
| activity cycle | diel (24 h) cycle of animal's activity pattern |
| activity of interest | 2 feeding activities: not feeding and feeding<br>3 burial states: buried, half-buried and exposed |
| resting | animal is inactive, meaning it has not moved for the last 5 minutes; this can occur whilst fully exposed, partially or fully buried |
| feeding | animal is actively feeding either on substrate or on walls; tentacles are exposed and head performs sweeping movements |
| burying/ emerging | animal is actively burying into or emerging out of the substrate |
| buried | animal is partially or fully buried into the substrate and inactive (see also 'resting') |
| burying cycle | diel (24 h) cycle of animal's burying pattern |
| excretion rate | rate at which animal excrete sediment (measured through dry weight of excreted sediment per 24 h) |

*Holothuria scabra*'s feeding activity can be somewhat independent of their burying cycle. Exposed animals are not necessarily feeding, while burying animals may still ingest sediment (Yamanouchi 1939, 1956; Wiedemeyer 1992; Mercier *et al.* 1999). While there are some conflicting reports in regards to the periodicity of feeding when the animals are exposed (Yamanouchi 1939, 1956; Wiedemeyer 1992; Mercier *et al.* 1999), authors agree on feeding cycles being pulsed and variable (Hamel *et al.* 2001; Purcell 2004), with some studies indicating temperature as a cue for certain feeding habits (Roberts *et al.* 2000). However, no study has investigated the relationship between temperature and feeding rates for adult *H. scabra*.

When addressing conservation and fishery management of *H. scabra*, an understanding of burying activity is crucial to minimise errors in population and distribution surveys. Additionally, seasonal variation in burying and feeding activity may affect ecosystem function and bioturbation rates attributed to holothurians within their habitat. The aim of this study was to investigate a possible relationship between burying/feeding activity and temperature, while excluding other possible factors that may influence the animal's burying and feeding pattern such as tides, current and light. The present study solely investigated burying and feeding behaviour under a decreasing temperature regime such as the animals might experience in the wild as autumn changes to winter.

## MATERIALS AND METHODS

A glossary of terms used throughout this paper is presented in Table 1.

### EXPERIMENTAL SET-UP

The experiment was carried out in a temperature controlled room at the Moreton Bay Research Station on North Stradbroke Island, Queensland.

Six aquaria were set up, each with 10 cm of muddy sand as the substrate. Based on results from previous studies and personal observations (Wiedemeyer 1992; Wolkenhauer unpub. data) this is an adequate sediment depth to allow for normal burying behaviour of adult *H. scabra*, since their anus is usually in constant contact with the water column to facilitate respiration.

**Table 2.** Activity of *H. scabra* in aquaria classed as combinations of burial state and behaviour.

| Behaviour burial state | Fully buried | Half buried | Fully exposed |
|---|---|---|---|
| resting | 1 | 2 | 3 |
| burying | | 4 | |
| emerging | | 5 | |
| feeding levelled (on substrate) | | | 6 |
| feeding upright (on walls) | | | 7 |
| searching | | | 8 |

The substrate was collected from the field at a shallow station where *H. scabra* is frequently visible (Myora Gutter, 27°27.876'S, 153°25.146'E). The sediment was neither sieved nor treated to keep natural food sources (micro flora and fauna) intact. Aquaria were filled with seawater and aerated.

Three artificial lamps (DegenPai 36W ATT BR-HG (UV bulb) and DegenPai 36W D-HG FL6500KT8 (daylight bulb)) were placed over the aquaria, each covering two aquaria to simulate natural summer light regimes (14 h light and 10 h darkness). At 10 cm water depth the light reading was 1200 lux or 100 fc, at 30 cm water depth (sediment-water interface) it was 450 lux or 36 fc. Temperature was set to 24°C. Each of the six aquaria was stocked with one adult sandfish (~17 cm length; ~300 g wet weight) collected from the above field location. The animals were left to acclimatise in the aquaria for two days before the experiment. Subsequently, temperature was decreased one degree every day for a week until reaching 17°C at the end of the experiment.

In addition, another 12 aquaria were set up in a wet laboratory as control animals for faeces collection in the same way as described above. The only difference to the aquaria set-up in the controlled room was ambient light (through ceiling flood lights and windows), and flow-through sea water at a constant ambient temperature of 24°C. The sampling design and data collection for ingestion rates of these animals was the same as the ones in the temperature controlled room and is described below.

SAMPLING DESIGN AND DATA RECORDING

We monitored aquaria every two hours for seven days and classified activity of the animals on each occasion as various combinations of burial state and behaviour (Table 2). Furthermore, faeces produced by each individual were collected two-hourly and weighed after each 24-hour period.

Two common activities of interest were established for the analysis of burying cycles: A) buried (score 1, 2, 4 and 5); and, B) exposed (score 3, 6, 7 and 8).

For the analysis of feeding activity, two activities of interest were also established: A) not feeding (score 1, 2, 3, 4, 5 and 8); and, B) feeding (score 6 and 7). We did not observe *H. scabra* ingest any sediment while stationary or searching, since oral tentacles were retracted, and these were considered resting/searching periods (score 3 or 8). Thus, only hours spent by the animals moving along the substrate/walls with oral tentacles extended were considered as feeding periods (score 6 and 7).

Two main approaches were used to characterise both burial state and feeding activity: i) the time of day of each animal's state within the diel cycle; and, ii) the average amount of time per day the animals spent in each state.

STATISTICS

Statistical analyses were done using R 2.5.0. In order to prepare the data for statistical analysis, we converted scores of different activities of interest into binomial form (true/false) and analysed the responses using a Generalized Linear Model (GLM) with binomial error structure. Each state, e.g. feeding/not feeding or buried/not buried, was therefore treated as a binary response and the probability of this behaviour occurring was estimated as a probability between 0 and 1. Furthermore, harmonic transformation of the time-of-day using sine and cosine functions, representing the daily feeding and burying cycles, were used as supplementary explanatory variables. A linear regression was used to analyse the correlation between temperature and excretion rates.
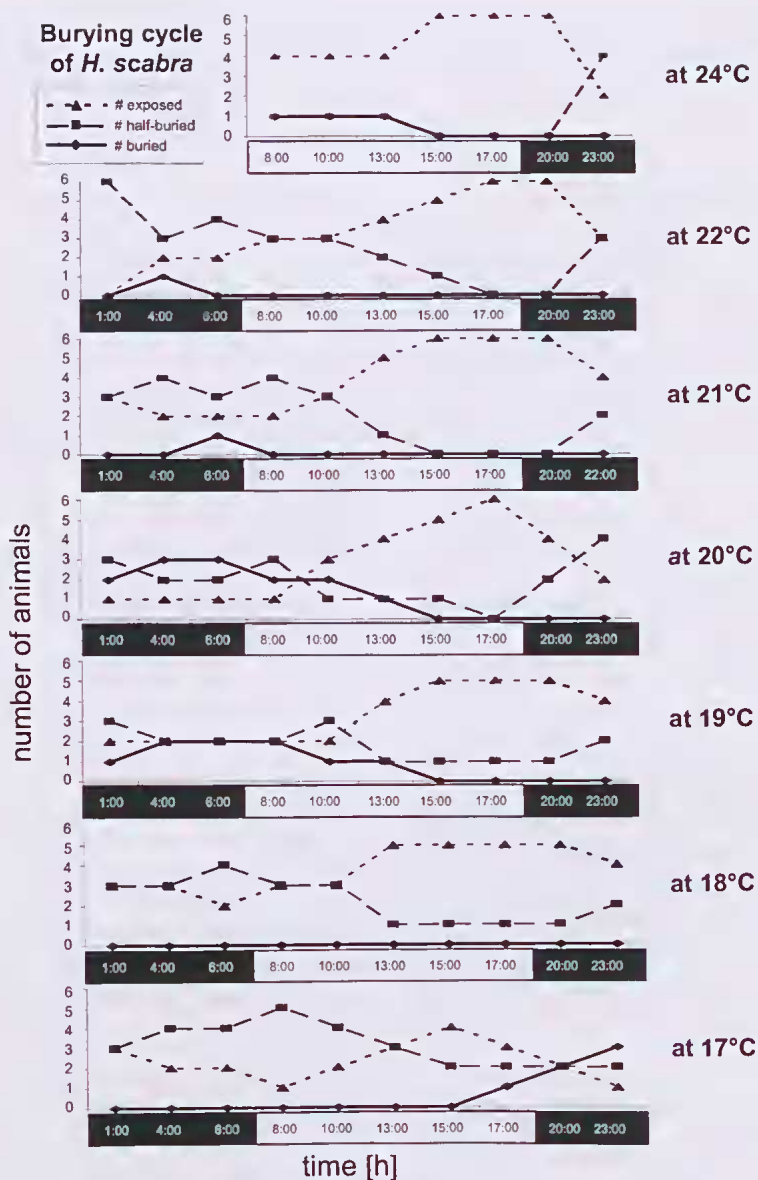
FIG. 1. Diel burying cycle of *H. scabra* with decreasing temperature. Open and solid bars on X-axis represent light and darkness.

## RESULTS

### TEMPERATURE EFFECTS ON BURIAL STATE

*Holothuria scabra* showed a distinct diel burying cycle (Fig. 1), with most of the animals exposed and active between the hours of 13:00 and 22:00 and most buried and inactive between the hours of 01:00 and 09:00. As experimental temperatures decreased, fewer animals spent time exposed and active, while more remained buried or half buried (Fig. 1). However, the trend of burying during the day did not change as such, but rather the burial duration lengthened.

The number of animals being buried (combining partially and fully buried) showed a significant (p = 0.002) negative correlation with
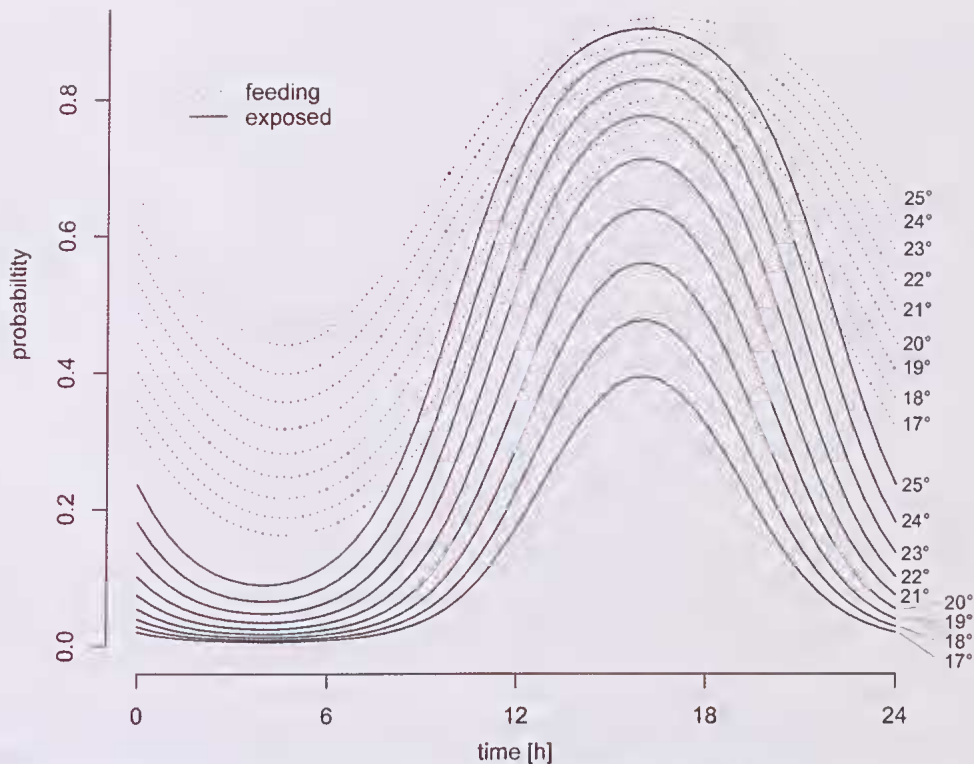
FIG. 2. Generalized Linear Model prediction of probability of feeding and burying behaviour (cos-sin function). Dotted line = probability of animals feeding, solid line = probability of animals being exposed. Numbers on the right represent the temperature in degrees celsius for each line.

temperature (Fig. 2, Table 3A). There was at least some period with the temperature above 20°C when no animals were buried, but a minimum of two out of six animals were buried at 17°C (Fig. 1). This effect was particularly obvious in the morning (08:00–10:00), with only one animal out of six being buried at 24°C, compared to four out of six animals being buried when temperature reached 17°C (Fig. 1).

*Holothuria scabra*'s average duration of being buried increased with decreasing temperature (Fig. 3A). When comparing combined values of being buried (fully and partially) against being exposed, periods being buried increased from 6.7 h at 24°C to 14.5 h at 17°C within 24 h.

### TEMPERATURE EFFECTS ON FEEDING ACTIVITY

There was a significant (p <0.001) correlation of feeding activity with temperature (Fig. 3B, Table 3B). Daily periods of feeding decreased by 9 h from 9.8 h at 24°C to about 0.8 h at 17°C. Especially during the last two days when temp-erature fell from 18°C to 17°C, a strong decrease in feeding activity was noticeable which might indicate a threshold temperature.

### TEMPERATURE EFFECTS ON FAECES PRODUCTION

Average daily faeces production decreased with decreasing temperature (Fig. 4), which formed a significant linear relationship ($r^2$ = 0.82, p <0.001, Table 4). The regression analysis indicated that there would be 5.7 g dry weight (DW) increase of faeces production with every degree of temperature up to 22°C. We also plotted the average faeces production from the twelve animals kept at a constant 24°C in flow-through aquaria. When we included data from two other studies, all results were close to the predicted regression line (Fig. 4).

### DISCUSSION

### BURYING CYCLES

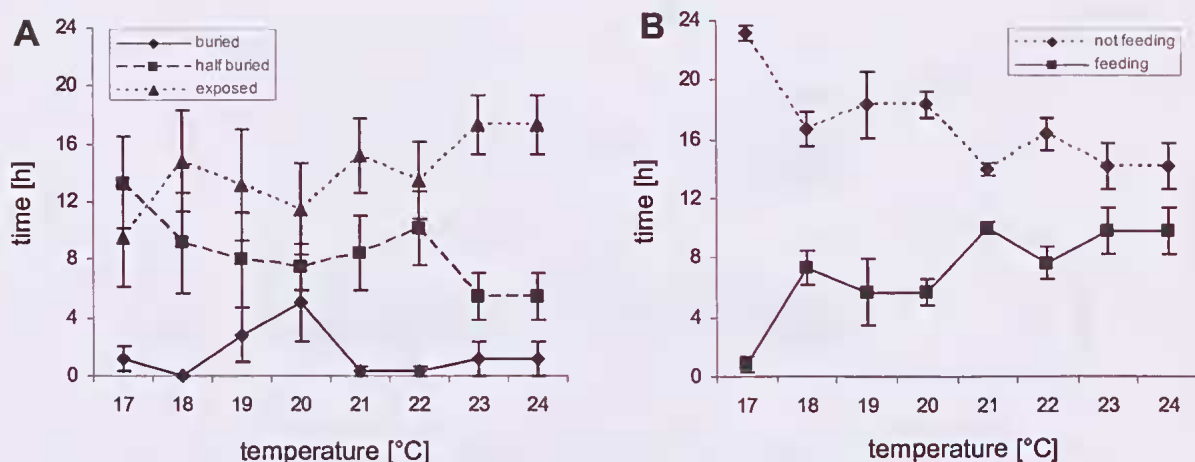This study shows that adult *Holothuria scabra* have a diel burying cycle as described for juv-

FIG. 3. Burying and feeding activity of *H. scabra* depending on temperature. **A.** Buried/half-buried versus exposed periods expressed as average hours spent in a day. **B.** Non-feeding versus feeding periods expressed as average hours spent in a day; error bars = 1 SE with n=6.

eniles (Battaglene *et al.* 1999; Mercier *et al.* 1999). Furthermore, the length of time spent buried shows a significant relationship to temperature. Purcell & Kirby (2005) also found more adult sandfish buried for longer periods during the day with decreasing water temperature. However, they did not specify any temperature range and did not investigate actual timeframes of the animals being buried based on a 24 hour-cycle since the observations took place only during daylight hours. Mercier *et al.* (2000a) found most

adult *H. scabra* on the surface did not follow their usual burying cycle when water temperature increased to more than 30°C.

There are other known factors that cause sandfish to bury for prolonged periods of time such as stress (Purcell *et al.* 2006), spring tides and strong current (Skewes *et al.* 2000), predation (Dance *et al.* 2003) and desiccation or changes in salinity (Mercier *et al.* 2000a). These factors might counteract or prolong the effect temperature has on their burying cycle in the

**Table 3A.** Results of GLM analyses of burial state (exposed/not exposed) in relation to temperature.

| Effect | Estimate | Standard error | z value | Pr (>|z|) |
|---|---|---|---|---|
| intercept | -3.270 | 1.172 | -2.791 | 0.005 |
| sin (t) | -1.266 | 0.162 | -7.802 | <0.000 |
| cos (t) | -0.462 | 0.153 | -3.027 | 0.003 |
| temperature | 0.175 | 0.057 | 3.075 | 0.002 |

**Table 3B.** Results of GLM analyses of feeding activity (feeding/not feeding) in relation to temperature.

| Effect | Estimate | Standard error | z value | Pr (>|z|) |
|---|---|---|---|---|
| intercept | -8.402 | 1.597 | -5.263 | <0.000 |
| sin (t) | -1.977 | 0.230 | -8.602 | <0.000 |
| cos (t) | -1.139 | 0.207 | -5.502 | <0.000 |
| temperature | 0.335 | 0.075 | 4.452 | <0.000 |

**Table 4.** Results of regression analyses of faeces production in relation to temperature.

| Effect | Coefficient | Standard error | t | P |
|---|---|---|---|---|
| intercept (constant) | -88.611 | 23.973 | -3.696 | 0.001 |
| temperature | 5.693 | 1.227 | 4.638 | <0.000 |

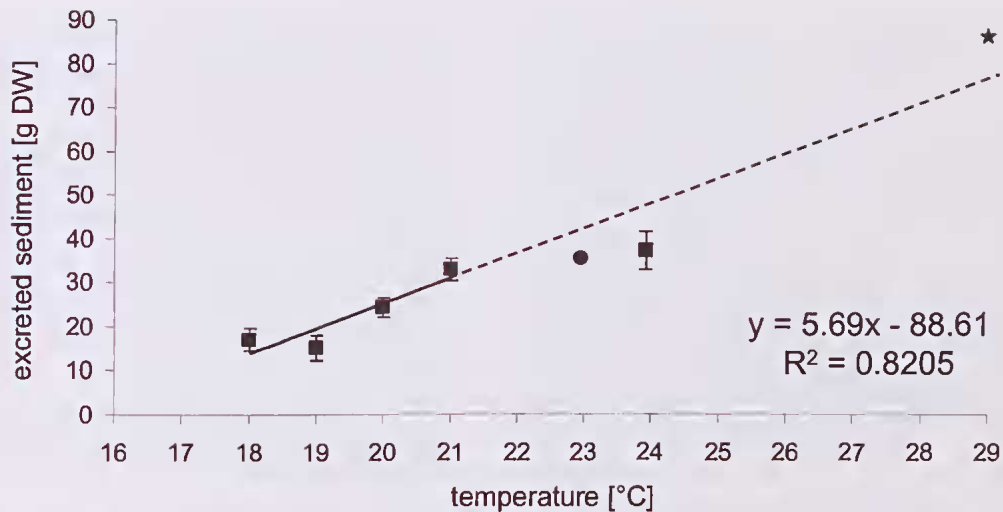$$y = 5.69x - 88.61$$
$$R^2 = 0.8205$$

FIG. 4. Solid line shows regression of faeces production of adult *H. scabra* (expressed in gram dry weight) versus temperature per 24-hour period. Error bars = 1 SE with n=6, d.f.=22. Dotted line is an extrapolation based on solid line. Three data points are added from other studies (but not included into analysis). Square represents 39.2 g faeces production at 24°C (control animals in this study), dot represents 38.4 g faeces production at 23°C (Purcell 2004) and star represents 88.8 g faeces production at 29°C (Mercier *et al.* 1999).

wild. However, this study aimed specifically to exclude those variable factors to find a potential underlying pattern in response to temperature alone.

Further study is needed to determine how light and temperature interact and if adult *H. scabra* have a potential tendency to reverse their burying cycle in accordance with reversed light regimes, overruling the temperature effect, as has been shown for smaller juveniles (Mercier *et al.* 1999).

### FEEDING CYCLES

Decreasing temperature has a significant effect on the animal's time spent exposed and feeding. The significant decrease in feeding behaviour between 18°C and 17°C (Bonferoni pair-wise comparison, p <0.001) supports a potential threshold temperature at 18°C for *H. scabra*. This is the usual winter minimum in Moreton Bay and sandfish do not occur any further south than this estuary (see distribution in Hamel *et al.* 2001), suggesting its southern distribution is temperature limited.

Studies on feeding behaviour of other echinoderms show similar effects with temperature (Schinner 1993; Hollertz & Duchêne 2001; Thompson & Riddle 2005). For example, Thompson & Riddle (2005) showed that the sea urchin *Abatus ingens* increased its displacement activity with increasing temperature.

### EXCRETION RATES

In the present study we show that the amount of faeces excreted by *H. scabra* has a significant positive relationship to temperature, and that our average excretion rate (33 g per 24-hour period at 22°C) correlates well with other studies (Mercier *et al.* 1999; Purcell 2004). Studies on ingestion rates of other echinoderms showed similar responses to decreasing temperature. For example, the heart urchin *Brissopsis lyrifera* decreased its ingestion rates from 1.92 g to 0.48 g dry sediment day$^{-1}$ when kept at 13°C and 7°C, respectively (Hollertz & Duchêne 2001).

Further investigation at higher temperatures is needed before a potential peak efficiency for food ingestion in relation to temperature can be estimated. *H. scabra* is a tropical species attaining its best growth rate and reproduction at water temperature ranging from 25–30°C (Hamel *et al.* 2001).

### CONCLUSION

Our data indicate that observed differences in burying and feeding behaviour of adult *Holothuria scabra* are strongly related to changes

in water temperature. These findings have implications for population surveys for this species, and related holothurians, when relying on visually counting animals during distribution and abundance surveys. Surveys should be conducted at consistent diel and seasonal timing if results are to be compared with previous data. Based on burying data presented here, we suggest the most suitable time to conduct population surveys on *H. scabra* would be during summer from midday to late afternoon. A reduction in ingestion rates at lower temperatures also indicates that bioturbation does not occur at equal rates during the year. Hence, the ecosystem function of holothurians is altered dependent on seasons and this needs to be taken into account when evaluating the ecological function of these animals within their habitat.

## ACKNOWLEDGEMENTS

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